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Remarks:
- This application was filed on 17-07-2000 as a divisional application to the application mentioned under INID code 62.
- Claims 11-68 and 70-72 are deemed to be abandoned due to non-payment of the claims fee (Rule 31 (2) EPC).

(54) A method of producing a taxane-type diterpene and method of obtaining cultured cells which produce the taxane-type diterpene at a high rate

(57) This invention relates to a method of producing a taxane-type diterpene(s) wherein tissues or cells of a plant which produces taxane-type diterpene(s) is cultured in the presence of at least one selected from the group consisting of jasmonic acids, compounds containing a heavy metal, complex ions containing a heavy metal, heavy metal ions, amines and antiethylene agents, a method of producing a taxane-type diterpene wherein the tissues or the cells of the plant are cultured by controlling the oxygen concentration in a gas phase in a culture vessel to less than the oxygen concentration in the atmosphere from the initial stage of the culture, or by controlling the dissolved oxygen concentration in a fluid medium which is in contact with the tissue or the cell to less than the saturated dissolved oxygen concentration at that temperature from the initial stage of the culture, a method of producing a taxane-type diterpene wherein the tissue or the cell of the plant is cultured in a culture vessel, while oxygenic gas containing 0.03 - 10 % of carbon dioxide is used as aeration gas to be introduced to the vessel, and a method of obtaining highly productive cultured cells for the taxane-type diterpene wherein cultured cells of the plant which produces the taxane-type diterpene are separated into a plurality of layers according to the difference in their specific gravities, and the cells contained in at least one layer are cul-

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ratio was 2.88. The results of the analysis of cells and medium taken out from the culture tank under stationary state showed that 525 mg of taxol was produced. That was equal to the productivity of 8.8 mg/liter/day.

[0293] The amount of the taxol contained in the cells and the medium were measured in the same manner as that used in Example 1.

[Example 80]

[0294] Fifty grams (fresh weight) of the same cultured cells that were used in Example 1 and 1 liter of liquid Woody Plant Medium were transferred to a culture tank (capacity of 2 liters) and the culture was carried out in the dark, at the agitation rate of 40 rpm, at 25 °C for 14 days, while an air to which 2 % carbon dioxide gas had been added was fed at 0.1 liter per minute. After completing the culture, the cells and the medium were collected and 15.2 g of dry cells were obtained. Determination of the amount of taxol contained in the cells and in the medium which was carried out in the same manner as that used in Example 1 showed that 31 mg of taxol was produced.

[Comparative Example 24]

[0295] The procedure of Example 1 was carried out except that jasmone was added instead of methyl tuberonate to give the final concentration of 0.1 - 1000 μM. The results are shown in Table 26.

[Comparative Example 25]

[0296] The procedure of Comparative Example 24 was carried out except that jasmone was not added. The results are shown in Table 26.

Table 26

	concentration of jasmone (μM)	cell yield (g/l)	yield*) of baccatin III (mg/l)	yield*) of taxol (mg/l)	yield*) of cephalomannine (mg/l)
Comparative Example 24	0.1	12.2	0.3	3.0	1.2
"	1	12.1	0.4	3.2	1.0
"	10	11.3	0.3	3.3	0.8
"	100	11.3	0.3	3.2	0.6
"	1000	10.9	0.2	2.5	0.8
Comparative Example 25	0	12.2	0.2	2.8	1.5

[*): The yield was calculated based on the total amount of production (in the cell + in the medium)]

Industrial Applicability

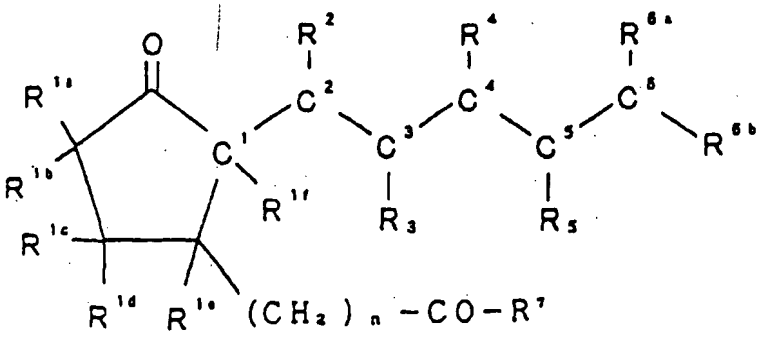
[0297] The present invention allows industrial production of a taxane-type diterpene including taxol which is useful as a therapeutic agent for ovarian cancer, mammary cancer, lung cancer and the like.

Claims

1. A method of producing a taxane-type diterpene wherein tissues or cells of a plant which produce taxane-type diterpenes are cultured in the presence of at least one selected from the group consisting of jasmonic acids, compounds containing a heavy metal, complex ions containing a heavy metal, heavy metal ions, amines and antiethylene agents, then the taxane-type diterpenes are recovered from the resulting cultures.

2. The method according to claim 1, wherein the culture is carried out in the presence of jasmonic acids.

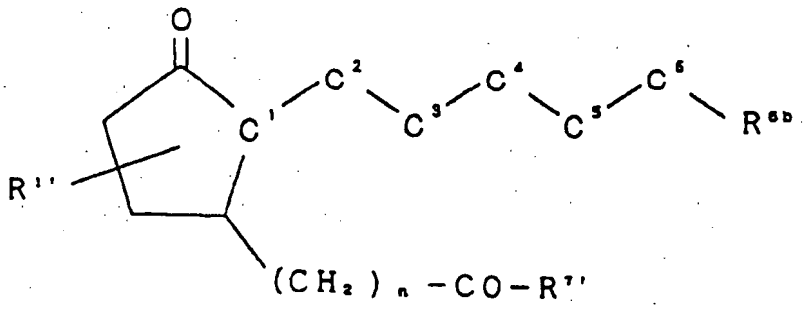
3. The method according to claim 2, wherein jasmonic acids are compounds represented by the general formula (I):



(I)

[wherein, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e} and R^{1f} respectively represent hydrogen atom, hydroxyl group, alkyl group having 1 to 6 carbon atoms, or alkoxy group having 1 to 6 carbon atoms;
R^2, R^3, R^4, R^5 and R^{6a} respectively represent hydrogen atom or alkyl group having 1 to 6 carbon atoms;
a side chain consisting of C^1- C^2- C^3 - C^4 - C^5 - C^6 may contain one or more double bonds;
R^{6b} represents hydroxyl group or -O- carbohydrate residue;
R^7 represents hydroxyl group, OM (wherein M is alkali metal atom, alkaline earth metal atom or NH_4), NHR^8 (wherein R^8 represents hydrogen atom, acyl group having 1 to 6 carbon atoms, alkyl group having 1 to 6 carbon atoms or amino acid residue), OR^9 (wherein R^9 is alkyl group having 1 to 6 carbon atoms or carbohydrate residue), or alkyl group having 1 to 6 carbon atoms;
n is an integer of 1-7;
and in the above-mentioned five-membered ring, a double bond may be formed between the neighboring member carbon atoms].

4. The method according to claim 3, wherein jasmonic acids represented by the general formula (I) are compounds represented by the general formula (I'):



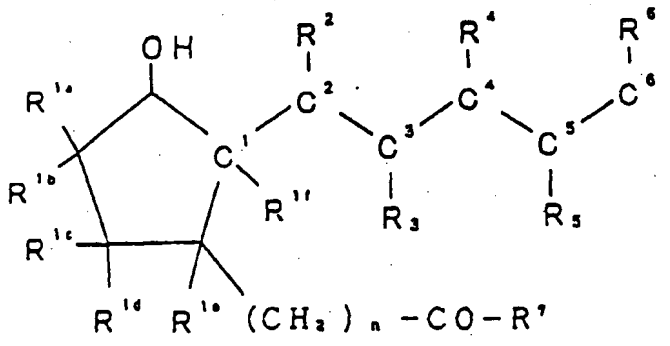
(I')

[wherein, $R^{1'}$ represents hydrogen atom or hydroxyl group;
a side chain consisting of C^1- C^2- C^3 - C^4 - C^5 - C^6 may contain a double bond between C^1 and C^2, between C^2 and C^3, or between C^3 and C^4;
R^{6b} represents hydroxyl group or -O- carbohydrate residue;
$R^{7'}$ represents hydroxyl group, OM (wherein M is alkali metal atom, alkaline earth metal atom or NH_4), $NHR^{8'}$ (wherein $R^{8'}$ represents hydrogen atom, acyl group having 1 to 4 carbon atoms, alkyl group having 1 to 4 carbon atoms or amino acid residue) or $OR^{9'}$ (wherein $R^{9'}$ represents alkyl group having 1 to 4 carbon atoms or carbohydrate residue);
n is an integer of 1-7;
and in the above-mentioned five-membered ring, a double bond may be formed between the neighboring

member carbon atoms].

5. The method according to claim 3, wherein a compound represented by the general formula (I) is tuberonic acid or methyl tuberonate.

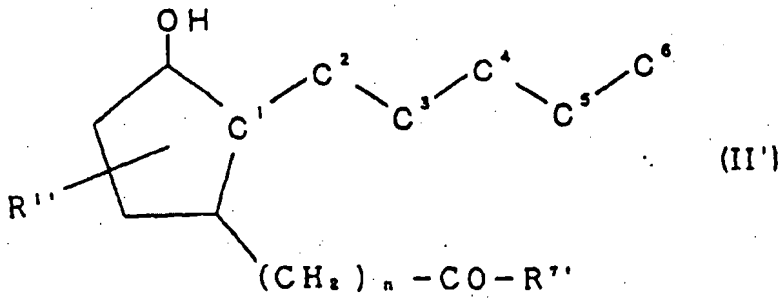
6. The method according to claim 2, wherein jasmonic acids are compounds represented by the general formula (II):



(II)

[wherein, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e} and R^{1f} respectively represent hydrogen atom, hydroxyl group, alkyl group having 1 to 6 carbon atoms, or alkoxy group having 1 to 6 carbon atoms;
R^2, R^3, R^4, R^5 and R^6 respectively represent hydrogen atom or alkyl group having 1 to 6 carbon atoms;
a side chain consisting of C^1- C^2- C^3 - C^4 - C^5 - C^6 may contain one or more double bonds;
R^7 represents hydroxyl group, OM (wherein M is alkali metal atom, alkaline earth metal atom or NH_4), NHR^8 (wherein R^8 represents hydrogen atom, acyl group having 1 to 6 carbon atoms, alkyl group having 1 to 6 carbon atoms or amino acid residue), OR^9 (wherein R^9 is alkyl group having 1 to 6 carbon atoms or carbohydrate residue), or alkyl group having 1 to 6 carbon atoms;
n is an integer of 1-7;
and in the above-mentioned five-membered ring, a double bond may be formed between the neighboring member carbon atoms].

7. The method according to claim 6, wherein jasmonic acids represented by the general formula (II) are compounds represented by the general formula (II'):



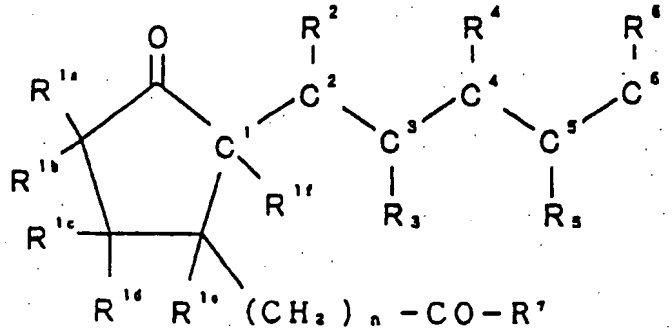
(II')

[wherein, $R^{1'}$ represents hydrogen atom or hydroxyl group;
a side chain consisting of C^1- C^2- C^3 - C^4 - C^5 - C^6 may contain a double bond between C^1 and C^2, between C^2 and C^3, or between C^3 and C^4;
$R^{7'}$ represents hydroxyl group, OM (wherein M is alkali metal atom, alkaline earth metal atom or NH_4), $NHR^{8'}$ (wherein $R^{8'}$ represents hydrogen atom, acyl group having 1 to 4 carbon atoms, alkyl group having 1 to 4 car-

bon atoms or amino acid residue) or $OR^{9'}$ (wherein $R^{9'}$ represents alkyl group having 1 to 4 carbon atoms or carbohydrate residue);
n is an integer of 1-7;
and in the above-mentioned five-membered ring, a double bond may be formed between the neighboring member carbon atoms].

8. The method according to claim 6, wherein a compound represented by the general formula (II) is cucurbic acid or methyl cucurbate.

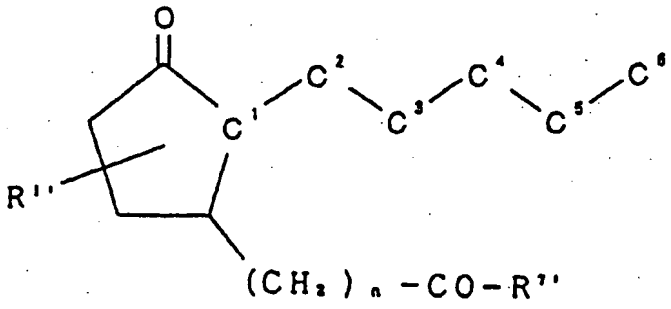
9. The method according to claim 2, wherein jasmonic acids are compounds represented by the general formula (III):



(III)

[wherein, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^{1e} and R^{1f} respectively represent hydrogen atom, hydroxyl group, alkyl group having 1 to 6 carbon atoms, or alkoxy group having 1 to 6 carbon atoms;
R^2, R^3, R^4, R^5 and R^6 respectively represent hydrogen atom or alkyl group having 1 to 6 carbon atoms;
a side chain consisting of C^1- C^2- C^3 - C^4 - C^5 - C^6 may contain one or more double bonds;
R^7 represents hydroxyl group, OM (wherein M is alkali metal atom, alkaline earth metal atom or NH_4), NHR^8 (wherein R^8 represents hydrogen atom, acyl group having 1 to 6 carbon atoms, alkyl group having 1 to 6 carbon atoms or amino acid residue), OR^9 (wherein R^9 is alkyl group having 1 to 6 carbon atoms or carbohydrate residue), or alkyl group having 1 to 6 carbon atoms;
n is an integer of 1-7;
and in the above-mentioned five-membered ring, a double bond may be formed between the neighboring member carbon atoms].

10. The method according to claim 9, wherein jasmonic acids represented by the general formula (III) are compounds represented by the general formula (III'),



(III')

[wherein, $R^{1'}$ represents hydrogen atom or hydroxyl group;

a side chain consisting of C^1- C^2- C^3 - C^4 - C^5 - C^6 may contain a double bond between C^1 and C^2, between C^2 and C^3, or between C^3 and C^4;

$R^{7'}$ represents hydroxyl group, OM (wherein M is alkali metal atom, alkaline earth metal atom or NH_4), $NHR^{8'}$ (wherein $R^{8'}$ represents hydrogen atom, acyl group having 1 to 4 carbon atoms, alkyl group having 1 to 4 carbon atoms or amino acid residue) or $OR^{9'}$ (wherein $R^{9'}$ represents alkyl group having 1 to 4 carbon atoms or carbohydrate residue);

n is an integer of 1-7;

and in the above-mentioned five-membered ring, a double bond may be formed between the neighboring member carbon atoms].

11. The method according to claim 9, wherein a compound represented by the general formula (III) is jasmonic acid or methyl jasmonate.

12. The method according to claim 2, wherein the concentration of jasmonic acids in a tissue culture medium is 0.01 - 1000 μM.

13. The method according to claim 2, wherein jasmonic acids are added when the cultured cells are in the exponential growth phase or in the stationary phase.

14. The method according to claim 2, wherein jasmonic acids are added to the culture medium in a plurality of parts or continuously.

15. The method according to claim 1, wherein the culture is carried out in the presence of at least one selected from the group consisting of compounds containing a heavy metal, complex ions containing a heavy metal and heavy metal ions.

16. The method according to claim 15, wherein the heavy metal is silver.

17. The method according to claim 16, wherein the compounds containing silver are at least one compound selected from the group consisting of silver nitrate and silver sulfate.

18. The method according to claim 16, wherein the compounds containing silver are at least one compound selected from the group consisting of silver fluoride, silver chlorate, silver perchlorate, silver acetate, silver sulfite, silver hexafluorophosphate(V), silver tetrafluoroborate, diamine silver(I) sulfate, and potassium diaminoargentate(I).

19. The method according to claim 16, wherein the complex ions containing silver are at least one ion selected from the group consisting of $[Ag(S_2O_3)_2]^{3-}$ and $[Ag(S_2O_3)_3]^{5-}$.

20. The method according to claim 16, wherein the complex ions containing silver are at least one ion selected from the group consisting of $[Ag(NH_3)_2]^+$, $[Ag(CN)_2]^-$, $[Ag(CN)_3]^{2-}$, $[Ag(SCN)_2]^-$, and $[Ag(SCN)_4]^{3-}$.

21. The method according to claim 16, wherein the concentration of compounds containing silver, complex ions containing silver or silver ion is 10^{-6}M - 10^{-1}M.

22. The method according to claim 16, wherein the concentration of compounds containing silver, complex ions containing silver or silver ion is 10^{-7}M - 10^{-2}M.

23. The method according to claim 15, wherein the heavy metal is cobalt.

24. The method according to claim 23, wherein the compounds containing cobalt are at least one compound selected from the group consisting of cobalt chloride, cobalt nitrate and cobalt sulfate.

25. The method according to claim 23, wherein the compounds containing cobalt are at least one compound selected from the group consisting of cobalt fluoride, cobalt perchlorate, cobalt bromide, cobalt iodide, cobalt selenate, cobalt thiocyanate, cobalt acetate, ammonium cobalt sulfate, cobalt(II) potassium sulfate, hexaamminecobalt(III) chloride, pentaammineaquacobalt(III) chloride, nitropentaamminecobalt(III) chloride, dichlorotetraamminecobalt(III) chloride hemihydrate, dinitrotetraamminecobalt(III) chloride, carbonatotetraamminecobalt(III) chloride, ammonium tetranitrodiamminecobaltate(III), sodium hexanitrocobaltate(III), tris(ethylenediamine)cobalt(III) chlo-

ride trihydrate, dichlorobis(ethylenediamine)cobalt(III) chloride, potassium tris(oxalato)cobaltate(III) trihydrate, potassium hexacyanocobaltate(III), potassium (ethylenediaminetetraacetato)cobaltate(III) dihydrate, hydridotetracarbonylcobalt(I), dicarbonyl(cyclopentadienyl)cobalt(I), octacarbonyldicobalt(O), hexacarbonyl(acetylene)dicobalt(O), bis(cyclopentadienyl)cobalt(I), and (cyclopentadienyl)(1,5-cyclooctadiene)cobalt(I).

26. The method according to claim 23, wherein the complex ions containing cobalt are at least one ion selected from a group consisting of pentaammineaquacobalt ion, nitropentaamminecobalt ion, dichlorotetraamminecobalt ion, dinitrotetraamminecobalt ion, carbonatotetraamminecobalt ion, tetranitrodiamminecobalt ion, hexanitrocobalt ion, tris(ethylenediamine)cobalt ion, dichlorobis(ethylenediamine)cobalt ion, tris(oxalato)cobalt ion, hexacyanocobalt ion, and (ethylenediaminetetraacetato)cobalt ion.

27. The method according to claim 23, wherein the concentration of compounds containing cobalt, complex ions containing cobalt or cobalt ion is 10^{-6}M - 10^{-1}M.

28. The method according to claim 23, wherein the concentration of compounds containing cobalt, complex ions containing cobalt or cobalt ion is 10^{-5}M - 10^{-2}M.

29. The method according to claim 15, wherein culture is carried out in the presence of jasmonic acids and at least one selected from the group consisting of compounds containing a heavy metal, complex ions containing a heavy metal and heavy metal ions.

30. The method according to claim 1, wherein the culture is carried out in the presence of amines.

31. The method according to claim 30, wherein the amines are polyamines.

32. The method according to claim 31, wherein the polyamines are at least one compound selected from the group consisting of putrescine, cadaverine, spermidine, spermin, ethylene diamine, N,N-diethyl-1,3-propane diamine, diethylene triamine and a salt thereof.

33. The method according to claim 31, wherein the concentration of the polyamines is 10^{-8}M - 10^{-1}M.

34. The method according to claim 31, wherein the concentration of the polyamines is 10^{-7}M - 10^{-2}M.

35. The method according to claim 30, wherein the culture is carried out in the presence of amines and jasmonic acids.

36. The method according to claim 1, wherein the culture is carried out in the presence of an antiethylene agent.

37. The method according to claim 36, wherein the antiethylene agent is a compound which inhibits the activity of an enzyme which catalyzes the conversion of S-adenosylmethionine into 1-aminocyclopropane-1-carboxylic acid.

38. The method according to claim 37, wherein the antiethylene agent is at least one compound selected from the group consisting of aminoxyacetic acid, acetylsalicylic acid, Rhizobitoxine, aminoethoxyvinylglycine, methoxyvinylglycine, α-aminoisobutyric acid, and a salt, an ester, an amino acid derivative and a carbohydrate derivative thereof.

39. The method according to claim 36, wherein the antiethylene agent is a compound which inhibits the activity of an enzyme which catalyzes the conversion of 1-aminocyclopropane-1-carboxylic acid into ethylene.

40. The method according to claim 39, wherein the antiethylene agent is at least one compound selected from the group consisting of gallic acid, and a salt, an ester, an amino acid derivative and a carbohydrate derivative thereof.

41. The method according to claim 36, wherein the antiethylene agent is a substance which removes the ethylene remaining in the cultures or existing in the gas phase or in the medium in the culture vessel containing the cultures.

42. The method according to claim 41, wherein the antiethylene agent is at least one compound selected from the group consisting of 1,5-cyclooctadiene, and isothiocyanic acid, a salt, an ester, an amino acid derivative and a carbohydrate derivative thereof.

43. The method according to claim 36, wherein the concentration of the antiethylene agent is 10^{-8}M - 10^{-1}M.

44. The method according to claim 36, wherein the concentration of the antiethylene agent is 10^{-7}M - 10^{-2}M.

45. The method according to claim 36, wherein the culture is carried out in the presence of an antiethylene agent and jasmonic acids.

46. The method according to claim 1, wherein the taxane-type diterpene is at least one compound selected from the group consisting of taxol, 7-epitaxol, baccatin III, 7-epibaccatin III, cephalomannine, 7-epicephalomannine, 10-deacetylbaccatin III, 10-deacetylcephalomannine, 10-deacetyltaxol, taxagifine, xylosyl cephalomannine, and xylosyl taxol.

47. The method according to claim 1, wherein the plant which produces the taxane-type diterpene is a plant belonging to genus Taxus.

48. The method according to claim 47, wherein the plant belonging to genus Taxus is at least one plant selected from the group consisting of Taxus baccata LINN, Taxus cuspidata SIEB. et ZUCC, Taxus cuspidata SIEB. et ZUCC var. nana REHDER, Taxus brevifolia NUTT, Taxus canadiensis MARSH, Taxus chinensis, and Taxus media.

49. The method according to claim 1, wherein cells of the plant which produces the taxane-type diterpene are fractionated into a plurality of layers according to the difference in their specific gravities, and cells contained in at least one layer are cultured.

50. The method according to claim 1, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by controlling the oxygen concentration in a gas phase in a culture vessel to less than the oxygen concentration in the atmosphere, from the initial stage of the culture, or by controlling the dissolved oxygen concentration in a fluid medium which is in contact with the tissues or the cells to less than the saturated dissolved oxygen concentration at that temperature, from the initial stage of the culture.

51. A method of producing a taxane-type diterpene wherein tissues or cells of a plant which produce a taxane-type diterpene are cultured by carrying out a two-stage culture, comprising a first stage using a medium to which an oxidizing agent or a water soluble organic compound containing oxygen is added and a second stage which is carried out according to the production method of claim 1, then the taxane-type diterpene is recovered from the resulting cultures.

52. The method according to claim 1, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by inoculating the tissues or the cells in a culture medium containing a saccharide in a concentration of 2 - 50 g/l,, and/or nitrate ion in a concentration of 2 - 50 mmol/l, then by adding a nutrient source solution containing the saccharide in an amount of 0.2 - 5 g/l, and/or nitrate ion in an amount of 0.2 - 5 mmol/l per day based on the initial volume of the said culture medium, continuously or intermittently to the culture medium, then the taxane-type diterpene is recovered from the resulting cultures.

53. The method according to claim 52, wherein the culture is carried out while the culture medium is renewed by adding the nutrient source solution and separating and removing the same volume of the culture medium from the tissues or the cells, and the taxane-type diterpene is recovered from at least one substance selected from the resulting tissues and/or cells, the culture medium recovered during the culture and obtained at the end of the culture.

54. The method according to claim 1, wherein a fresh medium is added continuously or intermittently in such a way that the specific renewing ratio defined by the dimensionless number $F = V_f/V/\mu$ (wherein, V is the total volume of the culture solution in a culture tank, V_f is the feed speed of the fresh medium, and μ is the specific growth rate of the tissues or the cells) is in the range of 0.1 to 10, and the taxane-type diterpene is recovered from the culture medium and the tissues or the cells contained in the culture medium which is continuously or intermittently taken out from the tank and/or from the culture medium containing no tissue and cell which is continuously or intermittently taken out from the tank.

55. The method according to claim 1, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by the use of oxygenic gas containing 0.03 - 10 % of carbon dioxide to be introduced to the culture vessel.

56. A method of producing a taxane-type diterpene wherein tissues or cells of a plant which produce a taxane-type diterpene are cultured by controlling the oxygen concentration in a gas phase in a culture vessel to less than the oxygen concentration in the atmosphere from the initial stage of the culture, or by controlling the dissolved oxygen concentration in a fluid medium which is in contact with the tissues or the cells to less than the saturated dissolved oxygen concentration at that temperature from the initial stage of the culture, then the taxane-type diterpene is recovered from the resulting cultures.

57. The method according to claim 56, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by controlling the oxygen concentration in the gas phase in the culture vessel to 4 - 15 %, or by controlling the dissolved oxygen concentration in the fluid medium which is in contact with the tissues or the cells, to 1 - 75 % of the saturated dissolved oxygen concentration at that temperature.

58. The method according to claim 56, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by controlling the oxygen concentration in the gas phase in the culture vessel to 6-12 %, or by controlling the dissolved oxygen concentration in the fluid medium which is in contact with the tissue or the cell, to 10 - 75 % of the saturated dissolved oxygen concentration at that temperature.

59. The method according to claim 56, wherein the tissue or the cell of the plant which produce the taxane-type diterpene is cultured by controlling the oxygen concentration in the gas phase in the culture vessel, or the dissolved oxygen concentration in the fluid medium by adjusting the oxygen concentration in aeration gas to be supplied to the culture vessel and/or to the culture medium, or by adjusting the feed speed of the aeration gas to be supplied to the culture vessel and/or to the culture medium.

60. The method according to claim 56, wherein the controlling of the oxygen concentration in the gas phase in the culture vessel or the controlling of the dissolved oxygen concentration in the fluid medium which is in contact with the tissues or the cells is started between the beginning of the culture and the 7th day after the start of the culture and the controlling is kept at least for 3 days.

61. The method according to claim 56, wherein the taxane-type diterpene is at least one compound selected from the group consisting of taxol, 7-epitaxol, baccatin III, 7-epibaccatin III, cephalomannine, 7-epicephalomannine, 10-deacetylbaccatin III, 10-deacetylcephalomannine, 10-deacetyltaxol, taxagifine, xylosyl cephalomannine, and xylosyl taxol.

62. The method according to claim 56, wherein the plant which produces the taxane-type diterpene is a plant belonging to genus Taxus.

63. The method according to claim 56, wherein the culture is carried out in the presence of jasmonic acids.

64. A method of producing a taxane-type diterpene wherein tissues or cells of a plant which produce a taxane-type diterpene are cultured by carrying out a two-stage culture, comprising a first stage using a medium to which an oxidizing agent or a water soluble organic compound containing oxygen is added and a second stage which is carried out according to the production method of claim 56, then the taxane-type diterpene is recovered from the resulting cultures.

65. The method according to claim 56, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by inoculating the tissues or the cells in a culture medium containing a saccharide in a concentration of 2 - 50 g/l, and/or nitrate ion in a concentration of 2 - 50 mmol/l, then by adding a nutrient source solution containing the saccharide in an amount of 0.2 - 5 g/l, and/or nitrate ion in an amount of 0.2 - 5 mmol/l per day based on the initial volume of the said culture medium, continuously or intermittently to the culture medium, then the taxane-type diterpene is recovered from the resulting cultures.

66. The method according to claim 65, wherein the culture is carried out while the culture medium is renewed by adding the nutrient source solution and separating and removing the same volume of the culture medium from the tissues or the cells, and the taxane-type diterpene is recovered from at least one selected from the resulting tissues and/or cells, the culture medium recovered during the culture and obtained at the end of the culture.

67. The method according to claim 56, wherein a fresh medium is added continuously or intermittently in such a way that the specific renewing ratio defined by the dimensionless number $F = V_i/V/\mu$ (wherein, V is the total volume of

the culture solution in a culture tank, V_l is the feed speed of the fresh medium, and μ is the specific growth rate of the tissues or the cells) is in the range of 0.1 to 10, and the taxane-type diterpene is recovered from the culture medium and the tissues or the cells contained in the culture medium which is continuously or intermittently taken out from the tank and/or from the culture medium containing no tissue and cell which is continuously or intermittently taken out from the tank.

68. The method according to claim 56, wherein the tissues or the cells of the plant which produce the taxane-type diterpene are cultured by the use of oxygenic gas containing 0.03 - 10 % of carbon dioxide to be introduced to the culture vessel.

69. A method of producing a taxane-type diterpene wherein tissues or cells of a plant which produce a taxane-type diterpene are cultured by the use of oxygenic gas containing 0.03 - 10 % of carbon dioxide to be introduced to the culture vessel, and the taxane-type diterpene is recovered from the resulting cultures.

70. A method of obtaining highly productive cultured cells for taxane-type diterpene, wherein cultured cells of a plant which produces a taxane-type diterpene are fractionated into a plurality of layers according to the difference in their specific gravities, and cells contained in at least one layer are cultured, then highly productive cultured cells for the taxane-type diterpene are selected from among those cultured cells.

71. The method according to claim 70, wherein the plant which produces the taxane-type diterpene is a plant belonging to genus Taxus.

72. The method according to claim 70, wherein cells contained in a layer having the specific gravity of 1.07 or less are cultured.